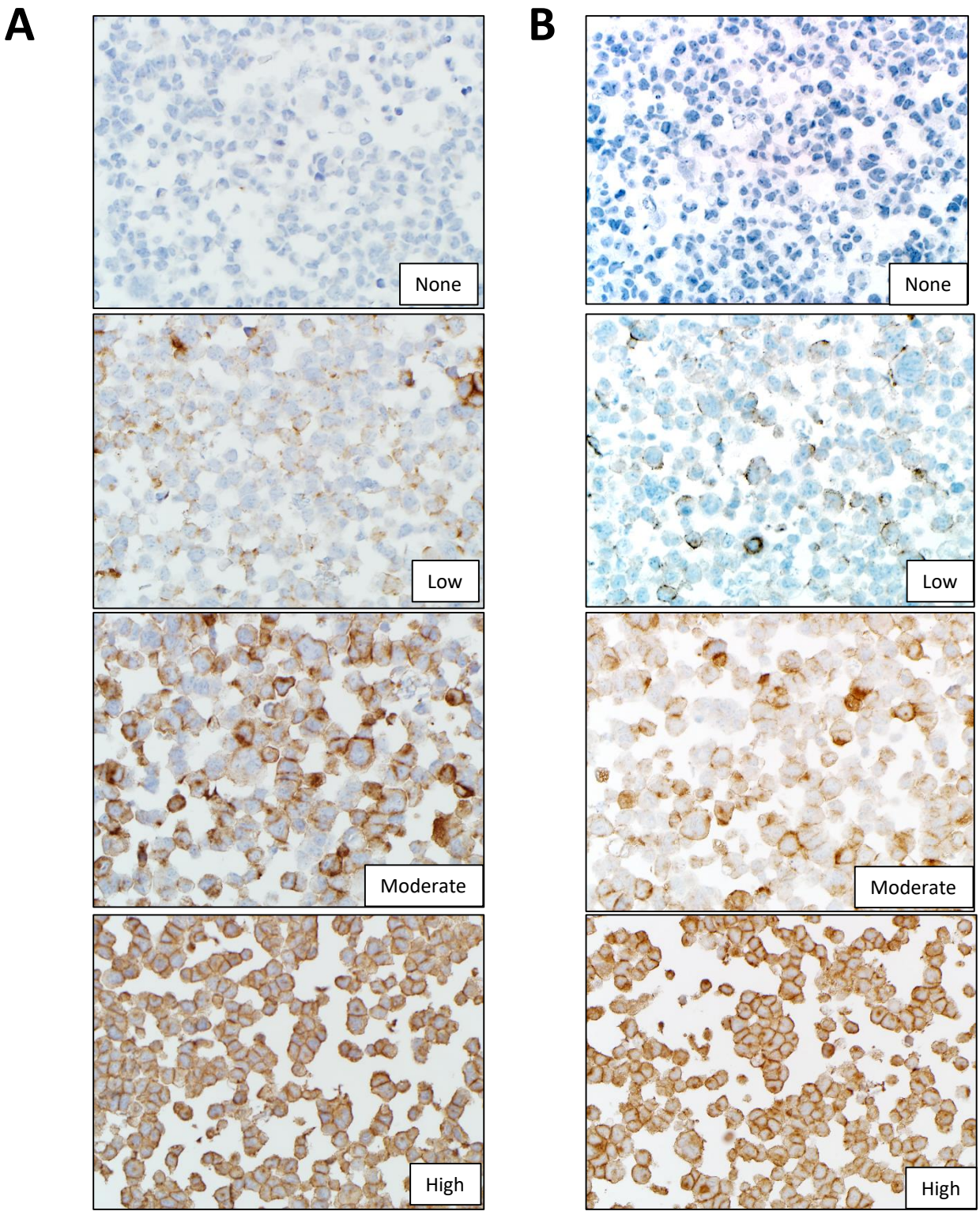
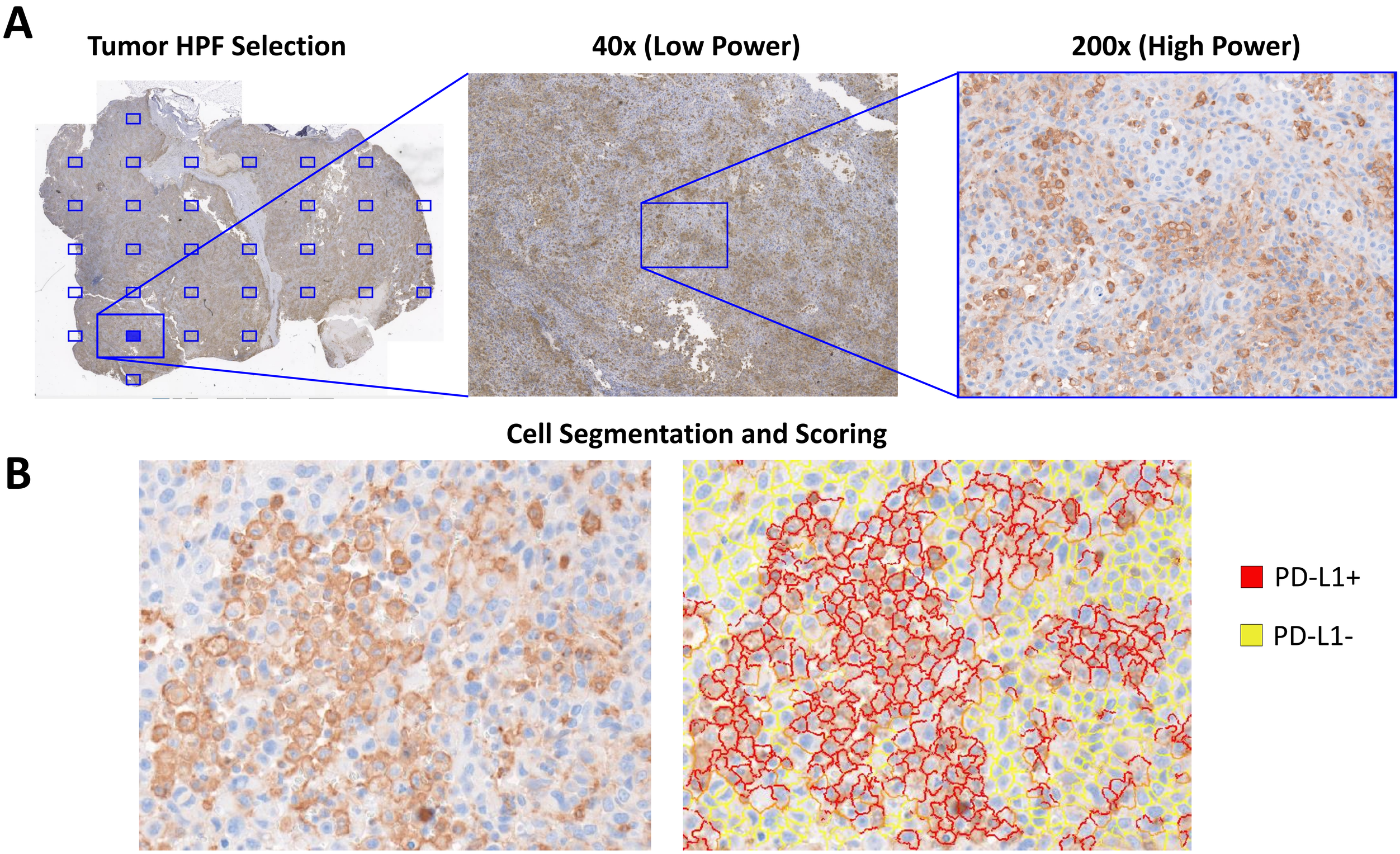
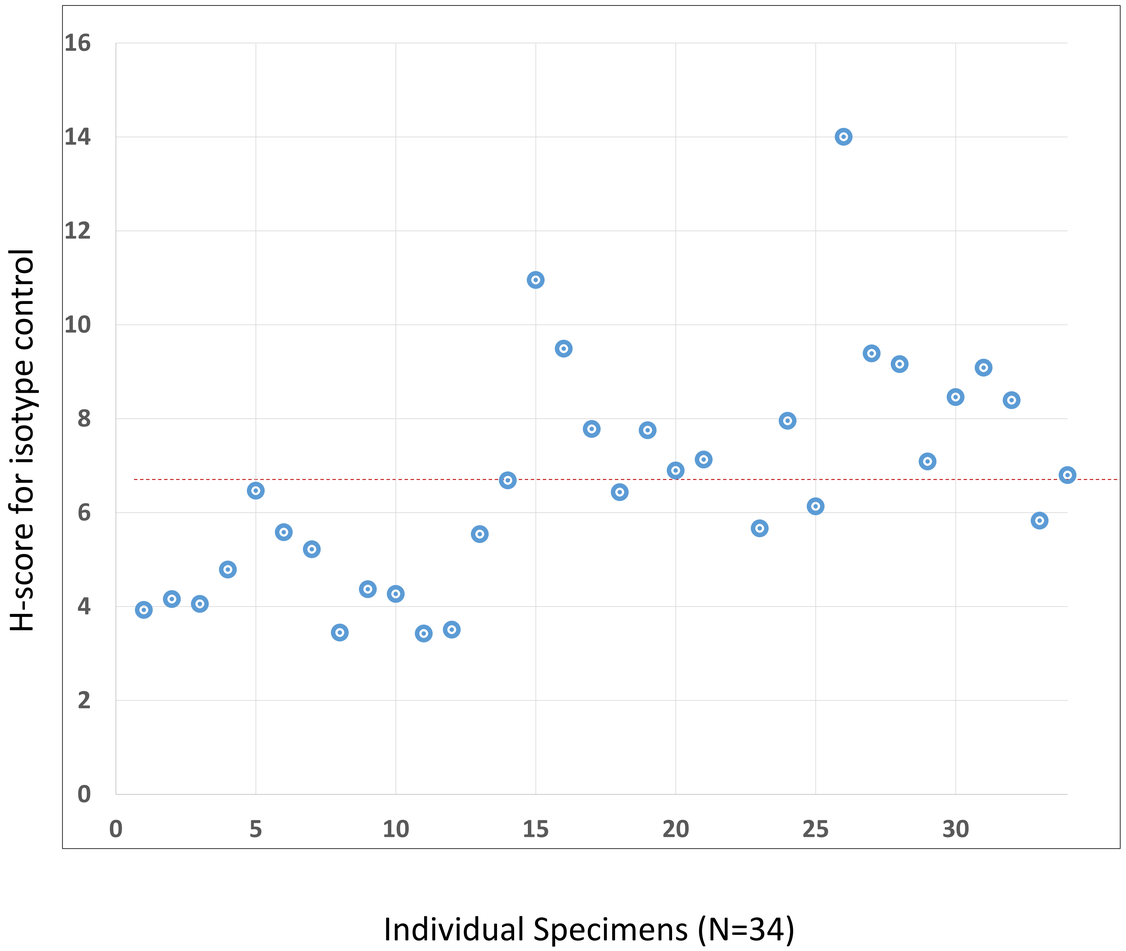


Supplementary Figure S1. Optimization of primary antibody concentration and antigen retrieval for PD-L1 IHC laboratory derived test using Horizon PD-L1 IHC reference standard. The reference slides contain an engineered cell line array with a range of protein expression levels (none, low, moderate, and high). Shown here are representative images for **(A)** the 5H1 clone and **(B)** the 22C3 clone. All other clones showed a similar staining patterns using the final assay conditions (data not shown). 400x original magnification, all panels.



Supplementary Figure S2. Computer-assisted imaging and scoring workflow. (A). Representative whole mount images (left panel), 40x (middle), and 200x (right) that were acquired of a metastatic melanoma specimen that was sampled at a frequency of 1:16. **(B).** Representative image at 500x (left panel) and resultant automated cell segmentation and scoring of membranous PD-L1 expression (right panel) shows the classification of PD-L1(+) cells by red outlines and PD-L1(-) cells by yellow outlines.

A.



B.

	5H1	SP142	28-8	SP263	22-C3
5H1		0.883	0.943	0.859	0.963
SP142	0.883		0.966	0.987	0.979
28-8	0.943	0.966		0.966	0.952
SP263	0.859	0.987	0.966		0.986
22-C3	0.963	0.979	0.952	0.986	

C.

	5H1	SP142	28-8	SP263	22-C3
5H1		0.808	0.838	0.829	0.949
SP142	0.808		0.909	0.910	0.885
28-8	0.838	0.909		0.921	0.916
SP263	0.829	0.910	0.921		0.877
22-C3	0.949	0.885	0.916	0.877	

Supplementary Figure 3. **Melanin in the cytoplasm does not impact the observed high concordances between PD-L1 antibodies**. The H-score of the isotype control largely reflects the amount of melanin within the tumor cells. **(A)** The cohort was divided at the median H-score (red dotted line) , and concordances were assessed for **(B)** cases with higher melanin content (n=16) and **(C)** cases with lower melanin content (n=18).

	Sampling Frequency						
Pathologist Score	1:25	1:25 inv	1:16	1:16 inv	1:9	1:9 inv	1:1
~30%	35.7%	35.6%	31.2%	30.1%	32.6%	32.0%	**31.9%**
~15%	18.0%	15.1%	16.0%	15.4%	14.8%	18.3%	**16.6%**
<5%	6.1%	7.4%	3.9%	2.2%	3.8%	3.3%	**3.4%**

Supplementary Table S1. Determination of sampling frequency for computer-assisted quantification of cell membranous PD-L1 expression. Full coverage high-power field imaging at 200x of an entire whole-mount tumor slide using the Vectra system results in very high data usage, with a 2 cm tumor requiring upwards of 500 200x fields and occupying over 75 GB of hard disk space. In an effort to determine if a lower imaging coverage would be sufficient to accurately assess the level of PD-L1 expression in the tumor, we optimized our sampling frequency, **Supplementary Figure S2**. Three melanoma specimens stained with SP142 were identified that had total cell percentage of PD-L1 expression ranges from 0 - ≤5%, 10 - 15%, and 25 - 30%, as assessed by pathologists on light microscopy. Automated image analysis was then used to assess the percentage of total cells demonstrating membranous PD-L1 expression at three coverage ratios (1:9, 1:16, and 1:25). Slides were then inverted, imaged, and scored again at the same ratios. Results were compared to the score obtained by 200x imaging of the entire tumor (listed under heading of 1:1 ratio in the table). A single representative case for each PD-L1 expression level is shown here. We found that sampling at 1:16 and 1:9 showed internally consistent results, as inverting and then resampling produced estimates of PD-L1 positivity that differed by only ~1% and was also within ~1% from the results generated when imaging the entire tumor.

	5H1	SP142	28-8	SP263	22-C3
5H1		0.946	0.925	0.949	0.960
SP142	0.946		0.938	0.968	0.907
28-8	0.925	0.938		0.962	0.903
SP263	0.949	0.968	0.962		0.884
22-C3	0.960	0.907	0.903	0.884	

Supplementary Table S2. **Concordances between PD-L1 antibodies using a single computerized scoring algorithm.** Cases deemed by the pathologists to have not been scored correctly by the initial algorithm and which required additional tuning (43/170) were excluded from this analysis.